Studies on the Development of the Veno-Lymphatics in the Tail-region of Polistotrema (Bdellostoma) stouti. First Communication: Formation of the Caudal Hearts.

By
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With Plates 19-21.

I. Introduction.

The purport of this and a following communication is to record certain facts and conclusions concerning the origin of the veno-lymphatics in the tail region of .Polistotrema (Bdellostoma) stouti. This paper will include a description of the adult condition of these vessels and the manner in which the caudal hearts are formed. Shortly I hope to follow with a second communication on the development of the veno-lymphatics in the tail region.

For several years I have been gathering together a series of Polistotrema embryos from Monterey Bay, California. Miss Julia B. Platt of Pacific Grove, California, Prof. G. C. Price of Stanford University, and Miss Julia Worthington of Cincinnati, Ohio, have generously added to my collection. All of the embryos were obtained by one Chinese fisherman, and unless someone was present to take charge of the material he was likely to preserve partly decayed embryos. Consequently a considerable portion of my material had to be discarded after sectioning; especially was this true of stages

between 15 and 20 mm. From 20 to 30 mm. and from 48 to 85 mm. my series are good and fairly complete.

My own specimens were all fixed in Tellyesnicky's potas-sium-bichromate-acetic mixture, embedded in paraffin, cut transversely 10 microns thick, and stained in either Heiden-hain's iron or Hansen's hæmatoxylin and counter-stained with a saturated alcoholic solution of orange G plus a little acid fuchsin. From my experience with the injection method I am satisfied that in many cases it would have only demon-strated the later stages of development, namely, the stage where the mesenchymal spaces had united to form a continuous canal, unless perchance there had been an extravasation of the injection mass, breaking down the mesenchyme separating these cavities, and possibly going still further. Upon inject-ing the lymphatics of fishes I have frequently broken down the delicate walls separating the lymphatics from a vein, filling the veins just as one sometimes fills the veins in the liver upon injecting the bile-vessels. In well-preserved material there is no reason whatever for regarding the mesenchymal spaces found in the region of growing lymphatics as artifacts, for if such, they would occur elsewhere in the mesenchyme.

My previous studies on the lymphatics of fishes have indi-cated that this system is more closely allied to the venous system than is the case in the higher vertebrates. This is especially true of Polistotrema, where these vessels always contained blood, although it should be stated that no direct connections were found with the arterial system. The primi-tive vertebrate doubtless had no distinct lymphatics other than the veins, which would also function for lymphatics, a portion of which in their later phylogeny may have differ-entiated into lymphatics. If this be true, it is reasonable to suppose that the lymphatics have the same origin, onto-genetically, as their more primitive ancestoral veins, of which considerable data shows that the larger veins do not arise as sprouts from a central venous centre, but rather grow from periphery to the centre, very much after the manner of the formation of the blood islands, mesenchymal spaces, in the

blastoderm, to form the omphalo-mesenteric vessels, and their endothelial walls are formed by a flattening of certain of the mesenchymal cells; while in a somewhat similar manner some of the enclosed mesenchymal cells are transformed by enlargement and rounding into red corpuscles. Prof. Scammon, in his recent work on Squalus, clearly shows from careful reconstructions that the anterior cardinals in this shark are laid down as isolated vessels before any communication is established with the venous part of the heart, and that this connection does not occur until a much later stage. In an early stage he finds a rather strong cephalic arterial connection, which he informs me likely gives rise to the anterior portion of this vein, but he is of the opinion that the posterior portion has an independent origin (probably from the union of certain mesenchymal spaces).

On account of the resemblance of the lymphatics to venous sinuses in Polistotrema I shall describe them as veno-lymphatics, which is indicative of a more generalized type, approaching the more primitive venous stage. It will be apparent that this use of the term differs from that made when it is applied to lymphatics arising ontogenetically from veins.

Notwithstanding the fact that the caudal heart and some of the caudal vessels of Myxine[1] and Polistotrema have been accurately described in the adult by Retzius, Klinckowström, Greene, and Favaro, it has seemed advisable for the sake of comparison to include here a brief description of these vessels as they appear in the adult from dissection and from serial transverse sections of a 20 cm. adult and an 85 mm. embryo that had assumed practically adult conditions.

II. Veno-Lymphatics in the Adult Polistotrema.

Caudal Hearts (Figs. 1 and 4, R. and L. Cau. H.).—Like Anguilla (eel), the caudal hearts are paired pulsating hearts

[1] A complete bibliography will be furnished in the next communication.

of considerable size, situated side by side, near the tip of the tail. They are nothing more than pronounced swellings of the anterior portion of the right and left forks of the caudal veins, which completely cover the lateral surfaces of the anterior third of the median ventral cartilaginous bar. At the point where the caudal veins (anterior portions of the caudal hearts) unite to form the common caudal vein their walls extend into the posterior end of the vein as valves, thus preventing a back flow of blood or lymph into the caudal hearts.

In the 20 cm. series the greatest caudo-cephalic diameter of the caudal heart is 2·52 mm., its greatest dorso-ventral diameter is 1·52 mm., and its greatest median-lateral diameter is ·1 mm. The corresponding measurements in the 85 mm. series are 1·1 mm. by ·47 mm. by ·1 mm. These measurements would indicate that the heart is about twice as long as it is high.

Both hearts in section (Fig. 1) have the general form of pyramids with their apices pointing dorsad. Usually both are filled with red corpuscles, but ordinarily one is distended more than the other. If Greene's observations are correct, that they contract alternately, it would account for there being more blood in one than in the other.

The inner wall of the heart is endothelium, surrounded by a layer of dense white fibrous tissue, which binds it inward to the median ventral cartilaginous bar and laterad to the musculi cordis caudalis. According to Favaro this layer abounds in elastic fibres, but none were visible in my sections, although it should be stated that I employed no specific stains. The musculi cordis caudalis (Fig. 1, *M. C. C.*), which functions as a partial myocardium, is attached anteriorly to the lateral process of the median ventral bar, and its striated longitudinal fibres, after passing posteriorly over the lateral surface of the caudal heart, become attached to the median ventral bar, and to a slight extent to the bases of some of the ventral or anal fin radials. This muscle later on will be shown to be more intimately related to the

myotomes of the great lateral or body-wall muscle than it is to the heart.

Concerning the innervation of the musculi cordis caudalis, in the 20 cm. and 85 mm. series there are four ventral spinal nerve rami that travel caudo-ventrad between the myotomes and the m. cordis caudalis and could innervate the m. cordis caudalis, but only from the third (counting from cephalo-caudad) were any fibres seen going to this muscle. The first of these nerves on the left side had its motor nucleus and its spinal ganglion considerably cephalad of the m. cordis caudalis or the caudal heart and slightly in advance of the forking of the caudal artery and vein. 'It travelled gradually caudo-ventrad, and in the region of the anterior end of the heart it passed between the myotomes and the m. cordis caudalis, slightly nearer the former, which it innervated ; but absolutely no fibres were seen going to the m. cordis caudalis. The motor nucleus and spinal ganglion of the second nerve was situated immediately dorsad of the passing of the first ventral ramus between the myotomes and the m. cordis caudalis. Like the first nerve it travelled caudo-ventrad between the myotomes and the m. cordis caudalis in the region of the anterior end of the caudal heart, but did not innervate the m. cordis caudalis. The nucleus or the third motor root and the spinal ganglion was dorsad of the crossing of the second ventral ramus between the myotomes and the m. cordis caudalis, and at the posterior end of the heart the ventral ramus of the third spinal nerve passed between the myotomes and the m. cordis caudalis, innervating both. In the case of the 85 mm. embryo the left caudal heart was in an embryonic condition at the point of innervation of the m. cordis caudalis by the ventral ramus of the third spinal nerve, consisting only of the original left caudal vein and one mesenchymal cavity some distance dorsad of it. The nucleus of the fourth motor root and ganglion was situated immediately dorsad of the innervation of the m. cordis caudalis, and, like the other ventral rami, the fourth had a caudal ventral course between the myotomes and the extreme caudal end of

the m. cordis caudalis, which is some little distance behind
the caudal heart, but, unlike the third ventral ramus, it inner-
vated only the myotomes.

The four ventral spinal nerve rami described above in
relation to the m. cordis caudalis are of considerable value as
landmarks for studying the limits of the embryonic caudal
hearts or in the adult they demonstrate that the m. cordis
caudalis extends over three segments, while the caudal heart
is somewhat shorter.

The peculiar state of the spinal cord in the region of the
spinal ganglion, resembling lateral ventricles of the central
canal, and an expansion of ·the central canal dorsad and
laterad in the form of rhombic lips, is reserved for a later
paper.

Most remarkable is the extremely rich blood supply in the
m. cordis caudalis of the 20 cm. series. Vessels of consider-
able size traverse long distances in this muscle, but their
connections outside the muscle are difficult to make out. It
can, however, be reduced to one artery and three veins if
the caudal heart is left out. The first or most anterior vessel
noticed in this region on the left side is an inter-segmental
vein, consisting only of a ventral or hæmal branch which
passes between the anterior portion of the m. cordis caudalis
and the myotomes, dorso-cephalad, to terminate in the caudal
vein a little in advance of the point of its bifurcation, and
so far as could be determined it received no branches from
the m. cordis caudalis. The second vessel is also a vein, but
it drains several segments and terminates in the left caudal
heart a little anterior of its centre. To trace backward, it
pierces the m. cordis caudalis and then continues gradually
caudo-ventrad between the m. cordis caudalis and the
myotomes to the posterior end of the heart, and it is this vein
that apparently collects the blood from the m. cordis caudalis.
A little anterior of the middle of the heart a typical inter-
segmental artery is given off from the lateral side of the left
caudal artery. It soon separates into dorsal and ventral
branches, and the latter travels caudo-ventrad with the second

ventral spinal nerve ramus, between the m. cordis caudalis and the myotomes, probably supplying both, though I could trace no branches going to the m. cordis caudalis. The fourth and last blood-vessel visible in this region is a vein, which terminates in the extreme dorso-lateral side of the left caudal heart, and to trace backward it passes over the m. cordis caudalis to the left caudal artery, which it follows for a short distance, and then divides into a large dorsal branch and a short ventral branch; the latter drains only the myotomes.

There is doubtless considerable variation concerning the arrangement of these inter-segmental vessels in different individuals, as will be shown in comparing the 20 cm. to an 85 mm. series, and also on the opposite sides of the same individual. In the 85 mm. embryo only three intersegmental vessels were noted. The first two were normal inter-segmental arteries and the third was a vein. The vein in many respects is homologous to the second vein of the 20 cm. specimen; its point of termination in the heart is not only much lower, but it is also much further caudad, being where the heart is still in embryonic condition.

According to Greene the caudal heart acts on the principle of a double force pump, the two m. cordis caudalis contracting alternately, and their rhythmic contractions are said to be governed by an automatic heart centre situated in the posterior end of the spinal cord.

Ventral Veno-lymphatic Trunk (Figs. 1, 2, 4 and 4 A, V. V. or R. and L. V. V.).—For convenience, and in part on embryological grounds, this important canal, which collects the lymph and blood from the entire tail region to discharge it into the caudal hearts, can be divided into two portions. First, an anterior portion (Figs. 1, 2 and 4, V. V. or R. and L. V. V.), which travels along the base of the anal fin immediately below the mucous sacs. Sometimes this trunk is a single canal and again it is paired; as a rule, it is a single vessel between two rays and paired opposite them. Hence, from a study of the adult alone one might erroneously take

the embryonic trunk to be a single trunk that later divided
in order to encircle a ray. At frequent intervals from either
side numerous connecting branches are received from each of
the lateral veno-lymphatic sinuses. Below and immediately
behind the lateral processes of the median ventral cartilagi-
nous bar this portion of the ventral trunk received the three
terminal branches of the posterior or transverse portion of
the longitudinal hæmal lymphatic trunks (Figs. 2 and 4,
L. Hæ. T. (1)), which is a short vertical trunk formed by the
posterior union of the two longitudinal hæmal lymphatic
trunks.

From this point caudad the remaining portion of the
ventral lymphatic trunk can be designated as the posterior
portion of the ventral trunk (Fig. 4), or possibly as the
caudal trunk from its similarity to the caudal lymphatic
trunk of the Teleosts. This canal, situated immediately
below the caudal hearts, traverses the ventro-lateral border
of the median ventral bar to its distal end, where it receives
a large anastomosing branch from each lateral sinus. Like
the anterior portion, it is a single trunk between two rays and
paired in the region of the rays. It also receives many com-
municating branches from the lateral sinuses. In the region
of the anterior portion of the caudal hearts one or two
branches (Figs. 1 and 4) are given off dorsad to communicate
with each caudal heart, and each orifice is guarded by two
valves opening into the heart (Fig. 1, *Val.* (1).) In the case
of a heart receiving but one communication from the posterior
portion of the ventral trunk, there was always noted in all
specimens examined a ridge in the floor of the heart where
one would expect the posterior communication with the
ventral trunk to occur; possibly this may represent fused
valve-folds that had formally guarded an orifice of an
embryonic ventral trunk communication.

Longitudinal Hæmal Lymphatic Trunks (Figs. 2, 3,
4, and 4A, *L. Hæ. T.* or *R.* and *L. Hæ. T.*).—Within the hæmal
canal there is a pair of large lymphatic canals situated on
either side of the caudal artery and between the caudal artery

and the caudal vein. Concerning their size, each exceeded
the calibre of the caudal artery. In the region of the lateral
process of the median ventral cartilaginous bar the paired
trunks unite in a common stem (Figs. 2, 4, and 4 A, *L. Hæ.
T.* $_{(1)}$), which soon bends ventrad between the two forks of
the caudal vein, and when immediately above the narrow re-
stricted portion of the ventral bar that is directly behind the
lateral processes it trifurcates. Two lateral branches (Fig. 4 A,
L. L. Hæ. T. $_1$) continue ventrad across the lateral
surface of the narrow restricted portion of the median
ventral bar immediately behind the lateral processes ;
while an anterior branch (Fig. 4 A, *A. L. Hæ. T.* $_1$) passes
at first cephalad and then ventrad to curve around in
front and between the lateral processes of the median ventral
bar, where it receives the ventral or inferior longitudinal
hæmal lymphatic trunk (Fig. 4 A, *V. L. Hæ. T.*), and at
this point of union there is a reservoir of considerable size,
which to a considerable extent in the 10 cm. series (but not
at all in the 85 mm. series) overlaps the sides of the lateral
processes. Immediately below the lateral processes and the
restricted portion of the median ventral bar these three
branches reunite in a common stem, which terminates in the
ventral veno-lymphatic trunk directly in front of the caudal
heart. A transverse section (as Fig. 3) sometimes shows one
of the longitudinal hæmal lymphatic trunks to be paired,
while another will show it single.

Throughout its course the longitudinal hæmal lymphatic
trunks receive at regular intervals the intersegmental
hæmal lymphatic trunks (Figs. 3 and 4 A, *Hæ. T.*) coming
up from between each set of myotomes. They always accom-
pany a corresponding artery or vein, sometimes lying in front
of it and again behind. As a rule between one pair of
myotomes there would be an artery and a lymphatic vessel,
and between the next pair a vein and a lymphatic vessel ;
there being, then, double the number of lymphatics as arteries
or veins, or the same number as arteries and veins
taken together. In case of some of the posterior hæmal

lymphatic vessels (see Fig. 4 A) these vessels were found to
terminate ventrad in the great lateral sinus, and on the level
with the top of the mucous sacs they sent inward a branch to
communicate with the ventral or inferior longitudinal hæmal
lymphatic trunk.

Ventral or Inferior Longitudinal Hæmal Lym-
phatic Trunk (Fig. 4 A, *V. L. Hæ. T.*).—Is a rather con-
spicuous vessel, though considerably smaller than the superior
longitudinal hæmal lymphatic trunk. It is situated midway
between the caudal vein and a longitudinal row of mucous
sacs. I have not studied this trunk anteriorly, but posteriorly
it terminated in the anterior fork of the common longitudinal
hæmal lymphatic trunk directly in front of the lateral pro-
cesses of the median ventral cartilaginous bar. The short
distance in which I have observed this trunk shows it to be
much larger posteriorly than it is anteriorly; hence the flow
of lymph is probably caudad. Small communicating vessels
have already been described coming from the intersegmental
hæmal vessels. The main branches received, however, are
segmental vessels that follow the surface of the anal or
ventral fin-rays between every two mucous sacs and commu-
nicate ventrad with the ventral veno-lymphatic trunk. These
vessels are not lettered, but are clearly shown in Fig. 4A, and
may be simply dorsal continuations of the ventral or anal fin-
ray vessels. Posteriorly in the 20 cm. series a direct connec-
tion with the superior longitudinal hæmal trunk was noted
together with several communications from a rather large
sinus situated on the lateral surface of the last two mucous sacs.

This reservoir (Fig. 4 A, *d.*) is quite conspicuous in the
10 cm. series, for it covers a large part of the lateral surface
of the last two mucous sacs. Dorsad it has at least two con-
nections with the inferior longitudinal hæmal lymphatic trunk,
cephalad it ends in rather loose lymphoid tissue, ventrad it
has several communications with the great lateral sinus, and
caudad it tapers down into a rather long stem that termi-
nates in the ventral veno-lymphatic trunk at the junction of
one of its connections with the lateral sinus. No similar

sinus was observed in the 85 mm. series, and how many of them occur in the 10 cm. series I am unable to state, since I have not sectioned an adult anterior of the last two mucous sacs.

Dorsal Veno-lymphatic Trunk (Figs. 1 and 4, *D. V.*).— Has much in common with the ventral veno-lymphatic trunk already described. In a transverse section (Fig. 1) it will be seen in a median line, between and a little above the level of the dorsal end of the myotomes. In the region of the caudal hearts it is situated directly above the median dorsal cartilaginous bar; immediately in front of the caudal hearts its position becomes more dorsad of this bar, and anterior of the dorsal fin it travels as a sinus in the centre of a connective tissue septum that joins the two great lateral muscles. Its cephalic termination I have not determined, but posteriorly it terminates, like the ventral trunk, at the distal end of the caudal carti- lage by sending off a large anastomosing branch to each lateral sinus. Also, like the ventral trunk, it is paired in the region of a cartilaginous ray and a single trunk between two rays, and throughout it sends off many connections to both lateral sinuses. The dorsal veno-lymphatic trunk is undoubtedly homologous to a similar trunk found in Scorpænicthys and Lepisosteus (Lepidosteus), more closely allied to the latter, but less significant, in that it does not receive branches from the dorsal fin; these in Polistotrema terminate in the lateral sinuses.

Lateral Veno-lymphatic Trunks or Sinuses (Figs. 1 and 2, *R.* and *L. L. S.*).—The enormous sinus-like condition of these trunks in Polistotrema is very different from any- thing found in the Selachians, Ganoids, or Teleosts, but is suggestive of the lymphatic sacs of the Amphibia. If the entire region drained by the lateral trunks and their inter- segmental branches in fishes was united in a common sinus covering the entire outer surface of the myotomes, and collecting the branches from the median fins, we would have a condition similar to that found in Polistotrema.

The limits of this sinus may be described as occupying

most of the space between the lateral body muscles and the
skin, and extending from one end of the body to the other.
Cephalad its ending has not been determined, but in the tail
it terminates blindly, immediately behind the caudal plate.
Dorsad and ventrad both sinuses have many communications
with the dorsal and the ventral veno-lymphatic trunks, and
through the latter its contents reaches the caudal hearts and
the caudal vein.   Ordinarily, in section (Fig. 1), one of the
lateral sinuses is much wider than the other, which is likely
due to the fact that it contains more blood or lymph.

Relatively there are not nearly as many corpuscles in the
lateral sinuses of the 20 cm. series as in the 85 mm. and still
younger embryos.   In the 85 mm. series one finds the con-
nective tissue in places outside the lateral sinuses completely
filled with red corpuscles, having the appearance of germina-
tive areas.

From all the median fins numerous sinus-like branches are
received by both lateral sinuses.   The so-called dorsal fin-
ray canals (Fig. 1, *D. S.*) come from the dorsal fins, the
ventral fin-ray canals (Fig. 1, *V. S.*) from the ventral or
anal fins, and the caudal fin-ray canals from the caudal
fins.   All of these vessels very closely resemble one another in
that they more or less envelop a fin-ray, traverse it from its
distal to its proximal end, and terminate in either the right or
the left lateral sinus.   Each gives off many branches to
anastomose with the adjacent fin-ray canals (Fig. 1), thus
forming a very coarse network of vessels in the fin, which, so
far as could be determined, had no connection with the arteries.

So far as was observed nearly all of the corpuscles found in
the veno-lymphatics and in the connective tissues outside were
red corpuscles, it being necessary to look some time to find a
leucocyte. In fishes, where death usually occurs after a violent
struggle, I have attributed the finding of red corpuscles in
the lymphatics to the rupturing of the delicate walls
separating them from the blood-vessels.   Prof. Carlson
informs me that an examination of the lymph of mammals
that have died from violent deaths also resulted in the finding

of blood in the lymphatics. But how account for the great number of red corpuscles found in embryos before any muscle-fibres were developed, and for older embryos and adults where a great proportion of the corpuscles were red ? It has already been stated that no direct corrections have been found between the arteries and the veno-lymphatics ; that the veno-lymphatics of the adult, especially the lateral sinuses, contain very few corpuscles compared to the late embryos, although the red greatly predominate ; that there are a multitude of red corpuscles apparently being formed in the connective tissue surrounding the lateral sinuses of the late embryos. Is it not, therefore, more than likely that in embryos the veno-lymphatics, especially the lateral sinuses, function as reservoirs or receptacles to hold the blood-corpuscles germi-nated in the nearby connective-tissue mesenchyme, and that the corpuscles migrate through the loose endothelial cells of the lateral sinuses, where many of them are seen undergoing cell division ?

There can be no question but that the dorsal, lateral and ventral veno-lymphatics and the caudal hearts of Polisto-trema are the homologue of similar structures found in Teleosts, Ganoids and Selachians.

III. Late Embryonic Condition of the Blood-Vessels.

In all of the good series, which began with two 20 mm. embryos, the caudal artery and vein were well formed, hence only a guess can be made at the manner of their formation. In the 27 mm. series, which was carried further cephalad than the others, I found in a section taken through the hind gut that the caudal vein was in direct connection with the splanchnic blood-vessels of the yolk-sac. This may indicate a similar origin of the two, and from my point of view would expect the splanchnic vessels of the yolk-sac to arise as they do in the chick blastoderm by the union of certain blood islands or mesenchymal spaces, and the transformation of the border mesenchyme into endothelium and the enclosed cells into red corpuscles.

In a very much decomposed embryo of 15 mm. the caudal artery and vein were present, but in a similar series of 10 mm. there was no trace of them.

As in the adult the caudal artery and vein in the 20 mm. embryo separated into right and left branches above a thickening of the mesenchyme that is forming the lateral processes of the median ventral cartilaginous bar. Each fork of the caudal artery (Figs. 5, 6, and 7, $R.$ and $L.$ $Cau.$ $A.$) passes caudad immediately under and to the side of the notochord, at first along the side and further caudad above the level of the embryonic median ventral cartilaginous bar. The two caudal veins (Figs. 5, 6, and 7, $R.$ and $L.$ $Cau.$ $V.$) follow at first obliquely ventrad across and then caudad along the ventro-lateral surface of the embryonic median ventral bar to a point in the 20 mm. series B (Fig. 6), 150 microns from the tip of the spinal cord or 290 microns from the tip of the tail. Here on the left side, and about the same place on the right side, the caudal vein and artery bend toward each other and unite, forming one continuous cross vessel on either side near the tip of the tail. Fig. 6 shows that there is in the anastomosing portion of the caudal artery and vein several large masses of mesenchyme ($P.$). These partitions were also found throughout the entire posterior portion of the caudal vein, including the region of the future caudal heart, of not only this 20 mm. series, but in another, series A, and in the 25 mm. series (Figs. 7 and 9, $P.$). This means, then, that this portion of the vein contains the remains of large mesenchymal cavity partitions that have not disintegrated at this stage. In the 25 and 27 mm. embryos the caudal artery and vein, on either side of the tail, were likewise found to be in direct connection a little cephalad of the ending of the notochord, but in all of the later stages this early connection is lost, and the caudal artery and vein extend further caudad and break up or receive small branches coming from, or going to, the caudal fin.

Laterad and ventrad of the points of union of the caudal artery and vein in the 20 mm. series there is nothing but

undifferentiated mesenchyme. No trace of the embryonic myotomes or lateral sinus mesenchymal spaces occurs until a section 210 microns cephalad of the tip of the spinal cord is reached.

Intersegmental Blood-vessels.—As previously stated in the adult under the head of the intersegmental or hæmal lymphatics there are twice as many intersegmental lymphatics as there are arteries or veins—that is, the arteries alternate with the veins in traversing the septa between the myotomes; while each septum would have a lymphatic vessel, it would have either an artery or vein, not both. A typical interseg- mental artery upon leaving the lateral side of the caudal artery almost immediately divides into a dorsal and ventral branch. In the course of the dorsal branch, it gives off lateral branches to the myotomes and a median branch to the neural canal; while the ventral branch passes ventrad along the inner surface of the septum between two myotomes, supplying each. The course of the intersegmental veins is practically the same as the arteries, except that the dorsal and ventral veins terminate separately at different levels on the lateral surface of the caudal vein instead of first uniting in a common stem. The veins, then, are apparently in a more primitive condition than the arteries.

It is not my intention to take up the development of these vessels only in so far as they are concerned in the formation of the caudal hearts, but in order to get a true conception or typical state of these vessels in the embryo it is necessary to examine them in front of the point of bifurcation of the caudal vessels.

In the 20 mm. series B, it will be seen at a glance that the intersegmental arteries are much further advanced than the veins, which have barely started. It will also be noted that the caudal artery and vein have assumed different shapes; the artery is very much depressed, having a very great lateral diameter and almost no dorso-ventral diameter, while the caudal vein is almost round. Throughout the whole length of the caudal vein there are on either side one or two

mesenchymal cavities, which for the most part have gained
connection with the vein and with each other. They are lined
with an undifferentiated mesenchymal endothelium, which cells
in many cases have lateral processes that cross these cavities as
well as line them. In a later stage, 27 mm., the mesenchymal
walls between these cavities and the caudal vein have broken
down, thereby greatly increasing the lateral diameter of the
vein. From these cavities both in the 20 and the 27 mm. series
short dosal and ventral intersegmental branches were given
off; these for the most part ended peripherally in more or
less of a cord of cells, indicating that the origin of the inter-
segmental veins is from sprouts off from the caudal vein.
Even in these small embryos the intersegmental arteries have
attained the course already described for the adult. In the
27 mm. embryo the median branch to the spinal cord is more
clearly defined than in the adult, and in both series the inter-
segmental arteries travelled much further laterad before
dividing than they did in the adult.

In the region of the caudal heart and behind the caudal
heart in the 20 mm. series A, which is not as far advanced as
series B, all of the intersegmental vessels are in an embryonic
condition. The caudal intersegmental vessels are more
numerous than intersegmental vessels should be; those in the
caudal heart region appear to be about twice the normal
number and to increase as you go caudad. Nowhere in this
region do you find a typical intersegmental artery arising
from the lateral surface of the caudal artery and dividing into
dorsal and ventral branches, but rather each branch takes its
origin from dorsal or ventral surface of the right or left caudal
artery (Fig. 7, *Ints. A.*) and then passes latero-dorsad or latero-
ventrad. In the region of the caudal heart there are at least
three latero-dorsal outpocketings, which have every evidence
of being embryonic intersegmental veins (Fig. 7, *Ints. V.*).
They at first passed laterad, then dorsad, and upon reaching
a height about on a level with the left caudal artery, but con-
siderably laterad of it, they end blindly. The second of these
embryonic veins in the heart region (Figs. 7 and 14, *Ints. V.*)

anastomosed with a corresponding ventral intersegmental artery, and several such communications were observed further caudad. No embryonic ventral intersegmental veins were found in the caudal region. From the lateral reconstruction (Fig. 7) several of the larger mesenchymal spaces (C.) might readily be taken for embryonic dorsal intersegmental veins, but transverse sections demonstrate their position and course to be too far mesad to be considered as such.

In the 20 mm. series B, 25 mm., 27 mm., and 60 mm. series there are no embryonic intersegmental veins in the region of the caudal heart, nor is there any evidence in these series of a degeneration of the embryonic veins shown in the 20 mm. series A. Also there is absolutely no reason for supposing that the degenerating endothelium of these intersegmental veins formed the cavities (C. and c.), in the reconstructions 6 to 10, for not only is their position and irregular arrangement against it, being located too far laterad, but instead of these spaces in the mesenchyme being lined with flattened endothelium they are bordered by undifferentiated mesenchyme. In the 20 mm. series B, 25 mm. and 27 mm. series these intersegmental veins have survived behind the caudal heart and are numerous. Posterior of the caudal heart in the 20 mm. series B, the embryonic intersegmental veins resemble the embryonic intersegmental veins of the heart region in the more embryonic 20 mm. series A, and in several places the veins were seen to anastomose with the arteries. Nowhere in the caudal region of the 20 mm. series B were any embryonic intersegmental veins given off ventrad; though in the 27 mm. series they were in the process of formation, apparently as sprouts from the caudal vein. In all of the embryos these embryonic intersegmental veins had at first a lateral course, and then bent dorsad or ventrad, being situated some little distance from the notochord, for the most part too laterad, to take any part in the formation of the so-called caudal heart mesenchymal spaces (C. and c.). No trace of well-formed intersegmental veins emptying directly into the heart, as have already been described for the adult, were seen until the

85 mm. series was reached.   Here in the extreme posterior
ventral corner, a ventral intersegmental vein pierced the m.
cordis caudalis and terminated in the left caudal heart through
a very small orifice.   This vein I took to be the homologue of
a more anterior vein in the 20 cm. series, which emptied into
the anterior end of the heart at about its median line.   This
would indicate, then, that the permanent intersegmental veins
emptying into the caudal hearts are of late formation,
beginning somewhere in embryos of between 60 and 85 mm.
in length.

As regards the intersegmental arteries in the caudal heart
region, many of them in the 20 mm. series B, 25 mm., 27 mm.,
and 60 mm. series have retained their primitive condition, as
in 20 mm. series A, of having dorsal and ventral intersegmental
arteries, arising separately from the caudal arteries; while in
some instances, as in the 27 mm. embryo, the basal parts of
these arteries have united in a common stem (Fig. 10, *Ints. A.*).
This part of the process of the development of the inter-
segmental arteries of Polistotrema may be, then, somewhat
analogous to the spinal ganglion cells in the mammalian
embryo changing from bipolar to unipolar as development
advances.

IV. Development of the Caudal Hearts in Polistotrema.

In considering the mode of formation of the caudal hearts
in Polistotrema the condition of the caudal hearts in each
of the various stages will be discussed, beginning with the
youngest.   Of these there are two well-preserved embryos of
20 mm. in length, which have been designated as series A and
B ; although of equal length, practically all of the structures
are considerably more advanced in series B.

20 mm. Polistotrema Series.

In both series A and B, the caudal hearts are shown by
lateral reconstructions of the left embryonic caudal heart
(Figs. 6 and 7) and by several transverse sections (Figs. 11 to

14) to be in very early stages of formation. In fact, the caudal vein itself may not have received its maximum size in this region any more than it has further cephalad or caudad, but since in the adult the caudal hearts are nothing more than large expansions of the anterior portion of the right and left branches of the caudal vein, it is impossible in the embryo to discriminate or define any boundaries between the enlargement of the caudal vein such as has been described both anterior and posterior of the caudal hearts from the normal development of the caudal hearts, for both take place in exactly the same manner.

A glance at the embryonic caudal heart in the reconstructions 6 and 7 demonstrates that the original left caudal vein ($L.$ $Cau.$ $V.$) is bounded, more or less on all sides, by certain mesenchymal spaces, from the region of the embryonic lateral processes of the median ventral cartilaginous bar to a point designated by $Z.$, denoting the posterior extremity of the caudal heart as determined by its relation to the spinal nerves. These spaces can readily be separated into two distinct types: first, mesenchymal spaces ($C.$) that have gained connection with the caudal vein, and second, mesenchymal spaces ($c.$) that have not at this stage obtained connection with the caudal vein.

For the sake of maintaining a logical order the small isolated mesenchymal cavities ($c.$), that have not at this stage gained connection with the caudal vein, will be considered first. As will be shown later they are undoubtedly the first cavities to appear. In the two reconstructions they are found everywhere where the heart is in the process of formation, and in the extreme posterior end of the embryonic caudal heart in reconstruction 6, which is the region of the heart last to form, they were the only cavities observed. One of these cavities is shown in sections 11 and 13; the former is taken through an isolated cavity in the posterior end of the embryonic caudal heart and the latter is from the anterior end. In both cases these cavities are small, but well-defined, not artifacts, and are bounded simply by the adjacent

mesenchyme, not at this stage differentiated into flattened endothelium.

In certain areas, as may be designated by the red corpuscle (*R. C.*) in Figs. 12 and 13, there are places which appear to be the very beginnings of the vacuolation of the mesenchymal spaces just described. This process as shown from the examination of a number of isolated mesenchymal spaces is apparently nothing but a degeneration or atrophy of certain of the mesenchymal cell processes and the thickening of others to form the boundaries of the cavity ; while some of the enclosed cells may enlarge, become round and develop into red corpuscles before the mesenchymal spaces become connected with the caudal vein.

Concerning the mesenchymal spaces (*C.*) which have already gained connection with the caudal vein, there are several distinct stages illustrating different periods of growth of the larger cavities that take such a conspicuous part in the excavating of the caudal hearts out of the mesenchyme. Of these an early, middle, and a late stage will be described with considerable detail. All of these stages are shown in outline in the reconstructions 6 and 7, while some of them may be seen to advantage in transverse sections.

The earliest stage of one of these connected mesenchymal spaces (*C.*) is well illustrated by the dorsal cavity to the right in Fig. 12. When compared to the isolated mesenchymal space (*c.*) in Fig. 11 it will be found to be identical, except that the mesenchymal lining or undifferentiated endothelium of the vein has broken down, joining the cavity with the vein. Observe in Fig. 12 some of the remains of the original partition at the orifice of the mesenchymal space. The character of this orifice and all similar mesenchymal space orifices opening into the caudal vein is strongly against the hypotheses that these spaces are out-pocketings or sprouts from the caudal vein, as is also the character of their walls. Immediately to the left of the above described mesenchymal space in Fig. 12 there is a slightly larger but identical connected mesenchymal space shown, only in this section its

extreme distal end is shown, which is the region of future growth, and the manner of growth by the breaking down of the mesenchymal cell processes is evident from this section. Opposite line 12 in the reconstruction 6; the above cavity (C.) is shown to have attained anterior connection with the left caudal vein.

Two dorsal connecting mesenchymal cavities (C) in the reconstruction 7 are typical examples illustrating a median stage of the development of a larger mesenchymal cavity that later becomes a portion of a caudal heart. These cavities simply represent one of the earlier connected mesenchymal spaces described in the previous paragraph as having united with one or more distal isolated mesenchymal spaces, thereby considerably increasing the size of the former. It might be surmised from an examination of the lateral reconstruction 7 that these spaces are developing intersegmental veins or capillaries, but their position in transverse section is shown to be too far median for them. In fact similar cavities are found median of the caudal vein—a region never penetrated by capillaries or intersegmental veins.

The third stage of development of the large connected mesenchymal cavities that contribute to the formation of the caudal hearts is simply a more advanced state of the middle stage previously described. It might be said to consist of several isolated mesenchymal spaces that have united with a larger connected mesenchymal space at either or both ends, and which brings these cavities in direct communication with the right or left caudal vein. Such a stage as this, or even a more advanced one, where all of the mesenchymal spaces have coalesced in one large cavity which is in direct connection at both ends with the caudal vein, are shown in the reconstructions 6 and 7. In Fig. 13 one of these cavities (C.) is shown in section opening into the left caudal vein, and in Fig. 12 two of these cavities (C.) appear ventrad of the right and left caudal vein. Since they are cut through different planes they will illustrate very well two different areas of one of the large cavities in the third stage of development. In

the floor of the left caudal vein will be seen (Fig. 12, *C.*) one of these cavities opening into the vein ; observe that the orifice bears evidence of the breaking down of the partition separating the cavity from the vein. In tracing this cavity cephalad it was found to open into three smaller mesenchymal spaces almost identical to the three mesenchymal spaces (*C.*) of another cavity of the third stage, shown below the right caudal vein in the same figure, and when followed further cephalad the mesesenchymal spaces terminated in a large cavity that emptied into the left caudal vein from below. A little anterior of the cavity described above there is another large connected cavity of the third stage, not shown in any of the transverse section figures, but almost identical to the cavity previously described. It has, however, one striking difference, namely, the process of the union of its mesenchymal spaces has progressed further ; while the mesenchymal walls separating these mesenchymal spaces in the first cavity were quite thick, resembling the condition found in the ventral cavity below the right caudal vein in Fig. 12 ; in this more advanced cavity they are reduced to two parallel mesenchymal cell processes, separating three median-lateral mesenchymal spaces from each other. These two remaining processes consist of the union of two ventral processes from two mesenchymal cells, now situated as border-cells on the dorsal side of the future mesenchymal cavity with two similar dorsal processes from two mesenchymal cells located on the ventral border of this future mesenchymal cavity ; while from all of these mesenchymal cells there are median-lateral processes, which will help constitute a sort of un-differentiated endothelial lining for this cavity. It would require but the atrophy of these delicate protoplasmic pro-cesses to convert the three mesenchymal spaces into one large mesenchymal cavity that would be connected at both ends with the left caudal vein, and in like manner the disintegra-tion of the partition separating this cavity from the vein would result in the excavation of a considerable portion of the caudal heart in the ventro-cephalic region.

Now the question might be asked, What becomes of the mesenchymal cells in the walls of the mesenchyme separating one space from another, as, for example, in Fig. 12, the ventral mesenchymal spaces (C.), when they coalesce to form one large cavity? It is quite certain that some of these cells are becoming round and increasing in size preparatory to becoming red corpuscles, while the border cells are apparently flattening to a slight extent in order to become endothelium for the caudal heart. It is doubtful, however, if all the cells take one or the other of these two courses. There remains, then, the processes of disintegration and migration as possibilities.

In connection with the process described above of an isolated mesenchymal space becoming in a later stage connected with the caudal vein or becoming a connected mesenchymal space as it was styled, the question might be asked, How do you know that this change actually occurs without being able to demonstrate it experimentally? In reply it might be said that it would be absurd to consider these well-defined mesenchymal spaces as artifacts, for if artifacts, they would occur in the mesenchyme outside the limits of the developing caudal hearts, blood-vessels, and lymphatics. In the immediate region of the caudal heart they are found only in the region of the small connected mesenchymal spaces and the larger mesenchymal cavities that unite with each other and the caudal vein to form the caudal heart. At the orifices of a great many of the small connected mesenchymal spaces and the larger mesenchymal cavities there are left the remains of the partitions that once separated these spaces or cavities from the vein, which is evidence against the hypothesis that they arose as sprouts or out-pocketings from the vein, as is also the fact that these cavities are lined with mesenchyme and not differentiated endothelium. Not many of these cavities have sufficient regularity to be the remains of intersegmental veins. In fact, they occur mesad of the caudal vein, a region never traversed by intersegmental veins or capillaries. The isolated mesenchymal spaces and the more

distal or growing areas of the larger mesenchymal cavities
apparently furnishes us with the solution of the origin and
manner of growth of these mesenchymal spaces and cavities.
Here in section may be seen the remains of what has tran-
spired. The protoplasmic processes of the mesenchymal cells
in the centre were found to be disintegrating and increasing
in diameter on the border of the cavity, while some of the
enclosed mesenchymal cells had increased in size, and were
becoming spherical to form the red corpuscles, and others in
the walls of the larger cavities had flattened in places to form
a part of the lining for the future caudal heart.

Another possible source of a very small portion of the
caudal hearts in Polistotrema is shown in the 20 mm.
series A to be from the proximal ends of three embryonic
intersegmental veins (Fig. 7, *Ints. V.*). These embryonic vessels
have already been described under the head of intersegmental
veins, and no trace of these vessels was found in the 20 mm.
series B or any of the later series until the 85 mm. series was
reached, indicating that the persisting intersegmental veins
of the adult did not arise in the caudal heart region until a
stage midway between 60 and 85 mm. The anterior and
posterior of these so-called embryonic intersegmental veins in
the 20 mm. series A (Fig. 7, *Ints. V.*) are in every way com-
parable to the intersegmental veins that are forming anterior
and posterior of the caudal heart region. Tracing them from
the dorso-lateral surface of the left caudal vein they pass
laterad for some little distance, and then bend dorsad to end
blindly, about on the level with the left caudal artery. If, then,
these are true embryonic intersegmental veins, arising as
sprouts from the left caudal vein, it would be possible for
their proximal portions to contribute slightly to the forma-
tion of the left caudal heart. Concerning the middle vessel,
which has been described as an embryonic intersegmental
vein anastomosing with a corresponding intersegmental artery,
is shown in transverse section (Fig. 14, *Ints. V.*) to bend dorsad
much sooner than the other embryonic intersegmental vein
did. It is possible, then, that this vessel is an embryonic

intersegmental vein that had been intercepted in its growth by the crossing of a ventral intersegmental artery, or it may have been only a capillary, or possibly a caudal heart mesenchymal cavity that had gained connection both with the caudal vein and an intersegmental artery. At any rate, granting it to be an embryonic intersegmental vein, it could have but little to do, relatively, with the formation of the left caudal heart. The mesenchymal space (Fig. 14, *C.*) opening into the so-called embryonic intersegmental vein has every indication of being an isolated caudal heart mesenchymal space that has acquired connection with the so-called embryonic intersegmental vein, rather than being a sprout from the vein. It will be seen, then, that the intersegmental veins or capillaries, which, according to Lewis, Baetjer, Huntington, and Miller, take such an important part in the formation of the lymphatic hearts in mammals, birds, and reptiles, takes but a very insignificant, if any, part in the formation of the caudal hearts in Polistotrema.

Both of the reconstructions 6 and 7 demonstrate that the left embryonic caudal heart has at this stage received no communication from the ventral veno-lymphatic trunk. In series B, reconstruction 6, the ventral veno-lymphatic trunk is represented by at least one large mesenchymal cavity (*l. v. v.* $_{(1)}$), which is about to gain connection with the embryonic caudal heart through the breaking down of the thin wall separating them. Also but little has been accomplished toward the differentiation of an endothelial lining, other than the thickening of the cell-processes lining the cavity and the slight elongation of some of the cells; it would be extremely difficult, if not impossible, to identify one of the typical dorsal or ventral border cells of the heart cavity from a typical mesenchymal cell. There is a marked massing of the mesenchyme to form the connective-tissue layer of the heart, but no differentiation of mesenchyme into connective tissue.

25 AND 27 MM. POLISTOTREMA SERIES.

A glance at fig. 9 will demonstrate that the left embryonic caudal heart (*l. cau. h.*) of the 25 mm. embryo has made considerable progress over both of the 20 mm. embryos. The anterior ventral region has been completely excavated, as has also the anterior dorsal region, except in the latter there is left traces of the mesenchymal partition that formally separated two of the larger cavities from each other or a cavity from the vein. The middle and posterior portions of the heart have not received their maximum dorsal growth at present, and the mesenchyme above and to either side of the original left caudal vein is invested by a number of large connected mesenchymal cavities and several isolated cavities, while the extreme posterior end of the caudal heart, namely, that portion located in front of the line *Z.*, is made up solely of the original caudal vein surrounded on all sides by isolated mesenchymal spaces (*c.*).

Anteriorly in the dorsal two thirds of the 25 mm. embryonic caudal heart the endothelium is much further differentiated than in the previous series, which means that its cells are somewhat more flattened, and their distal processes that formally extended out into the mesenchyme are lost, but ventrad, throughout the entire heart the border-cells are nearly round, very abundant, with almost no processes, and in a state where they could be easily moved. It would be impossible to discriminate between the ventral border-cells of the heart and the undifferentiated mesenchyme more distad.

There are no intersegmental veins emptying into the 25 mm. caudal heart unless the extreme proximal or caudal vein portion is considered as part of the heart ; if so, the caudal heart would receive a dorsal and a ventral intersegmental vein (Fig. 9, *Ints. V.*). It will be seen that the first or anterior mesenchymal cavity of the left embryonic ventral veno-lymphatic trunk (Fig. 9, *l. v. v.* $_{(1)}$) has gained connection with the anterior ventral corner of the left caudal heart, but no valves have appeared at the orifice (*O.*). Three other mesenchymal

cavities belonging to the left ventral trunk have also appeared in a row behind the first (Fig. 9, *l. v. v.* $_{(2)-(4)}$), and above the third and fourth, which have coalesced, the mesenchyme is vacuolating, indicating the place ($O._2$) where the second or posterior communication with the heart is likely to occur.

Reconstruction 10, which is from a 27 mm. embryo, shows a marked advance in the development of the caudal heart over the previous 25 mm. embryo as seen in reconstruction 9. Anteriorly the caudal heart had reached practically adult conditions so far as relative size was concerned, but from its centre caudad it consisted of a number of very large connected mesenchymal cavities (*c.*), which have occurred and assumed lines of growth in two different directions. The most anterior path or chain of these mesenchymal cavities had assumed a general caudal direction from the dorsal central part of the heart; while the posterior chain of the mesenchymal cavities had taken a dorsal course, considerably posterior, from the left caudal vein. A glance at Fig. 10 will show that the distal ends of these two different lines of growth of mesenchymal spaces, for the most part connected, were about to anastomose, and when this is accomplished the general outline of the heart will be completed, excepting that its dorsal boundaries will be expanded somewhat, and it will be added to posteriorly by the formation and union of a few additional mesenchymal spaces. These posterior isolated cavities were not present in this series, but were found in all of the later stages. As a result of the meeting and union of these two chains of mesenchymal cavities decribed above, there would be left in the centre an island of mesenchyme, identical to an anterior island (Fig. 10, *P.*) already formed, which in due time would be eliminated from the heart. From the lateral reconstruction 10, the posterior chain of mesenchymal cavities has the appearance of a developing intersegmental vein, but transverse sections reveal its position to be too far median— in fact it arises from the dorsal surface of the vein, and its course is dorsad close to the embryonic median ventral cartilaginous bar. Had not earlier stages of the caudal heart

region been examined, these two chains of more or less con-
nected mesenchymal cavities might readily have been taken
for out-buddings from the vein and the caudal heart, and the
caudal heart been described as being formed from sprouts
from the caudal vein.

The endothelium and connective-tissue layers were found
to be in about the same state as in the previous 25 mm.
embryo and no intersegmental veins terminated in the left
caudal heart.   Immediately below the anterior end of the
left caudal heart the two anterior mesenchymal cavities of the
left embryonic ventral veno-lymphatic trunk (Fig. 10, *l. v. v.*
(1), (2)) had united, forming a large cavity, which, as in the
previous series, had gained connection with the anterior
ventral corner of the heart, and about the orifice there was
a slight massing of the mesenchyme (Fig. 15, *val.* (1)), possibly
indicative of a very early stage in the formation of the valves
guarding this opening.   Likewise the third and fourth
cavities of the embryonic ventral trunk (*l. v. v.* (3), (4)) have
coalesced and the latter has joined the heart, thereby estab-
lishing the second or posterior communication between the
ventral veno-lymphatic trunk and the left caudal heart.

58 and 60 mm. Polistotrema Series.

As regards the caudal hearts in these two series, they
appear to be practically in the same stage, and since the
general preservation of the 60 mm. series is better, the des-
cription will be taken almost entirely from this series.

Except for a general dorsal expansion and a small caudal
addition, the caudal hearts of the 60 mm. series have not
increased in size relatively over the 27 mm. heart, but con-
sidering the general histogenesis of the organ, marked
progress has occurred.   The posterior embryonic condition
referred to above is in the posterior dorsal end of the heart,
where both isolated and connected mesenchymal cavities
(Fig. 8, *c.* and *C.*) are present.   Not only has the heart gained
anterior and posterior connections with the ventral veno-

lymphatic trunk, but conspicuous right and left valves (Fig. 16, *Val.* ₍₁₎) will be seen guarding the first or anterior orifice, and similar valves are in the process of formation at the opening of the second or posterior communication. The reason why these valves are not shown in reconstruction 8 is that the reconstruction is made through the median line of the heart, and it would therefore pass directly between the right and left valves.

Concerning the histogenesis of the layers of the heart at this stage, most any section—as, for example, Fig. 16—will demonstrate that the endothelium is well differentiated; instead of consisting of many rounded cells with rather short processes, as in the earlier series, we have here the characteristic flattened cells and long processes of the adult. Also the outer connective-tissue layer has changed from a concentrated mass of mesenchyme to a layer of fairly well differentiated fibrous connective tissue (Fig. 16, *F. C. T.*), and the m. cordis caudalis (Fig. 16, *m. c. c.*), although still in an embryonic state, is sharply marked out from the connective-tissue layer of the heart and the outer undifferentiated mesenchyme.

85 MM. POLISTOTREMA SERIES.

No figures are given to illustrate the condition of the caudal hearts in this series, which have attained an adult state, except for the extreme caudal end, which bears a striking resemblance to the 60 mm. hearts described above.

The valves and the different layers of the hearts strongly resembled the 20 cm. adult, and one intersegmental vein was found emptying into the posterior end of the left heart.

Since the rate of growth of the Polistotrema embryo is very slow, the time consumed from the beginning of the development of the caudal hearts in the 20 mm. embryo to their completion in the 85 mm. embryo, or a little later, requires, probably, several months. This, together with the primitive state of the lymphatics in the adult, should make Polistotrema a most favoured vertebrate for the onto-

genetic study of a veno-lymphatic heart. A striking difference between the caudal heart of Polistotrema and the embryonic sacs of the higher vertebrata is that the caudal hearts of Polistotrema, when once formed, never lose their primary connection with the vein, and acquire a second communication. It was shown in the adult to be a conspicuous swelling of the two posterior branches of the caudal, and to be formed after the manner outlined below.

From the previous description it will be seen that the main process involved in construction of the caudal hearts in Polistotrema consists, first in the formation of certain isolated mesenchymal spaces in the region of the anterior ends of the two branches of the caudal vein, by the breaking down of certain mesenchymal cell processes in the centre, and the thickening of others to form the boundaries of the cavity. Some of the cells in the centre may become spherical, increase in size, and eventually become transformed into red corpuscles. The next stage results in the breaking-down of the mesenchymal partition between this cavity and the caudal vein. About this time other more distal isolated mesenchymal spaces will occur. These will increase in size, meet and unite with the above-mentioned connected mesenchymal cavity, and frequently the distal ends of these cavities will coalesce in a larger cavity that will come in contact with, and join, the caudal vein by the disintegration of the partition separating them, thus establishing a second connection with the vein for above-mentioned cavities. While this is being accomplished, or shortly after, the mesenchymal walls separating the middle spaces will become broken down, leaving one large mesenchymal space that is connected at either end with the caudal vein. Apparently, in places the border mesenchymal cells of this cavity are flattening to contribute to the endothelium of the heart, while certain of the enclosed cells, as was noted for the isolated spaces, had, by increasing in size and becoming spherical, differentiated into red corpuscles. In like manner the mesenchyme on all sides of the anterior portion of the two forks of the caudal

vein becomes honeycombed with mesenchymal spaces and cavities of the various stages described above, beginning anteriorly and gradually occurring more posteriorly. By the coalescence of the larger cavities with each other and the vein, through the disintegration of the walls separating them, the caudal hearts are excavated out of the mesenchyme, mainly from above and below the anterior portion of the two branches of the caudal vein, and the caudal hearts for the most part become lined with endothelium derived from the flattening of the boundary mesenchymal cells of the larger cavities, and not from the migration of cells originating from pre-formed endothelium.

It was shown beyond a reasonable doubt that the isolated mesenchymal spaces were not artifacts, and that they, as well as the larger connected mesenchymal cavities, frequently occur mesad of the two branches of the caudal vein—a region never penetrated by capillaries or intersegmental veins.

In the 20 mm. series A, reconstruction 7, it was demonstrated that it would be possible for the proximal ends of three so-called dorsal intersegmental veins to take a minor part in the formation of the caudal heart. It should, however, be stated that no trace or remains of these embryonic vessels were found in the 20 mm. series B or in any of the later series until the 85 mm. stage was reached. Consequently there would be just as much reason for advocating that the border cells of these embryonic vessels disintegrated or possibly reverted back to mesenchyme, from which their cells are hardly distinguishable, as to maintain that in some way they contributed to the formation of the caudal heart. Granting, then, that they did take part in the formation of the Polistotrema caudal heart, it could only be a minor factor, and not the main process as is held by Lewis, Baetjer, Huntington and Miller for the lymph-sacs of mammals, birds and reptiles.

The formation of the caudal hearts as I have interpreted them in Polistotrema is almost identical to what Stromsten

found in the turtle, and in line with the earlier work on the lymphatics, as illustrated by Budge and Sala, but at variance with a considerable recent work on the development of the lymphatic sacs in the higher Vertebrata by Miss Sabin, Huntington, McClure, Lewis, Knower, Hoyer, Baetjer, Barański, and others, who maintain that the lymph-sacs in mammals, birds, reptiles and amphibians have their origin directly from the larger veins, either as sprouts or fenestrations, or from their branches or capillaries.

V. Development of the Ventral Veno-Lymphatic Trunk.

It is my intention in this paper only to take up the manner of formation of this trunk in so far as to isolate it from the caudal hearts, with which it is very closely associated.

In a transverse section taken through the anterior end of the left embryonic caudal heart of a 20 mm. series, as, for example, Figs. 5 and 13, there will be seen some distance below the left caudal vein a conspicuous isolated cavity in the mesenchyme ($l. v. v. _1$), containing several red corpuscles. If no further examination of this cavity be made, it might easily be dispensed with as an isolated ventral mesenchymal cavity that would take part in the formation of the left caudal heart. If, however, this cavity is traced further cephalad it will be found, as is shown in reconstruction ($6, l. v. v. _1$), to be situated too far ventrad to take any part in the construction of the left caudal heart, and in addition it can be followed under the embryonic lateral process of the median ventral cartilaginous bar to a point within 30 microns of a vertical plane that passes through the point of bifurcation of the caudal artery, which of course is considerably cephalad of the limits obtained by the caudal heart. It will also be noted that this rather large cavity was formed by the union of two or more smaller mesenchymal spaces. This large cavity I believe to be the first, or most anterior, of a series of mesenchymal cavities that will occur later and coalesce to form the posterior portion of the ventral veno-

lymphatic trunk. At this stage it has not obtained connection with the anterior end of the embryonic caudal heart, although transverse section 13 demonstrates that the mesenchyme between the two is becoming vacuolated, indicating the place where the first or anterior communication will occur. Still further caudad in reconstruction 6 there is another isolated mesenchymal cavity (v. v.), which occupies a position too ventrad to take part in the formation of the left caudal heart, and more than likely represents one of the more posterior cavities of the chain of cavities that will later unite in forming the posterior portion of the ventral trunk. The 20 mm. series A portrays a still earlier stage of this most anterior cavity of the ventral trunk, which is nothing more than a small vacuole in the mesenchyme (Fig. 7, $l. v. v._{(1)}$).

The left ventral veno-lymphatic trunk in the 25 mm. series is shown in reconstruction 9 to consist of a longitudinal chain of at least four mesenchymal cavities ($l. v. v._{(1)-(4)}$). The first cavity ($l. v. v., _{(1)}$) is of considerable size, and is probably derived from the union of several smaller mesenchymal spaces. As in the 20 mm. series, it not only passed cephalad under the embryonic left lateral process of the median cartilaginous bar, but extended further cephalad to a point 30 mm. in advance of union of the two branches of the caudal vein and considerably past the point of bifurcation of the caudal artery, while posteriorly it had gained connection with the anterior ventral corner of the left caudal heart, probably through coming in contact with the heart and the breaking-down of the wall separating them. Of the three remaining cavities, the first or second in the chain ($l. v. v._{(2)}$) is still isolated, while the two last have united in a single cavity, and the mesenchyme above this cavity is more or less vacuolated, preparatory to establishing the second or posterior connection ($o._{(2)}$) with the left caudal heart.

In the 27 mm. series, reconstruction 10, the embryonic ventral veno-lymphatic trunk is represented by a longitudinal chain of at least five mesenchymal cavities. The first and second cavities have coalesced in one, which not only com-

municates above with the caudal heart, but about the orifice
there is a slight massing of the mesenchyme (Fig. 15, *val.* $_{(1)}$)
possibly indicative of the beginning of valves, and the
anterior ending of this cavity under the embryonic lateral
process of the median ventral bar resembles the ending of the
first cavity of the 25 mm. series. The third and fourth
cavities (*l. v. v.* $_{(3), (4)}$) have also united, but the fifth
(*l. v. v.* $_{(5)}$) is still isolated. While the fourth cavity has gained
connection with the left caudal heart there is at present no
trace of valves at the orifice. Both the second and the fourth
cavities have joined two isolated mesenchymal cavities
(*L. L. S. C.*) belonging to the left embryonic lateral veno-
lymphatic sinus, and the third mesenchymal cavity has
gained direct connection with the left lateral sinus, doubtless
through a union of certain of the lateral sinus mesenchymal
spaces. It might be said for both embryonic ventral veno-
lymphatic trunks of the 27 mm. series that they consist of at
least five, more or less, connected cavities situated directly
below the caudal heart ; that they have obtained connection
with both the lateral sinuses and the caudal hearts, and these
vessels have now obtained a length equal to that of the
caudal heart.

Reconstruction 8 of the 60 mm. series shows the left ventral
veno-lymphatic trunk (*L. V. V.*) to have assumed practically
adult conditions in the region of the caudal heart, with the
exception that there will be found in a few places the remains
of former mesenchymal cavity partitions that have not com-
pletely disintegrated. The orifices of the anterior and
posterior communications between the ventral trunk and the
left caudal heart are guarded by valves, well developed in the
former and in the process of formation in the latter.
Numerous connections (*L. L. S. Con.*) have been received from
the left lateral sinus, and immediately in front of the caudal
hearts the three terminal branches of the posterior portion of
the longitudinal hæmal lymphatic trunk will be found to
culminate.

VI. FORMATION OF THE CAUDAL HEART VALVES.

As has already been pointed out in the 27 mm. series there is a slight massing of the mesenchyme (*val.* $_{(1)}$) around the orifice of the first or anterior communication between the left ventral veno-lymphatic trunk and the left caudal heart, which may be the forerunner of two valves that will occur later. It is not, however, until the 58 and 60 mm. series are reached that any real clue is obtained as to the manner of the formation of these valves.

In the 60 mm. series the right and left valves (Fig. 16, *Val.* $_{(1)}$) guarding the orifice of the first or anterior communication between the left ventral veno-lymphatic trunk and the left caudal heart are conspicuous and fairly well formed. They are composed of masses of mesenchyme or of but little differentiated connective tissue, which has migrated dorsad and inward into the heart from the right and left walls of the ventral trunk's connecting vessel. A close inspection of their structure will disclose that, notwithstanding that they belong to the same outer connective-tissue layer of the heart and ventral connecting vessel, yet their differentiation into connective tissue has not advanced nearly as far. The valves guarding the second or posterior communication of the ventral trunk with the left heart are in a much earlier stage of formation, in fact, only the very beginnings of embryonic connective-tissue valve-folds are visible on either side of the orifice. What appears to be an intermediate stage is shown at the orifice of the first or anterior communication of the ventral trunk with the right caudal heart. Here the right and left valve-folds of but little differentiated connective tissue from the side walls of the communicating vessel have grown inward and dorsad, and have fused irregularly, so that in places there would be a solid wall of tissue, and in other places temporary openings. Fig. 17 shows a region of these valve-folds where there is an opening on the left side, while in the next section cephalad (Fig. 18) there has been a complete fusion of the valve-folds, so that there is no orifice, and

in the next section cephalad a temporary opening is present, but on the right or opposite side to Fig. 17. Just how the permanent valves are constructed out of this rather coarse sieve-like valve-fold, or why these valve-folds should fuse at all, my material does not demonstrate, but would expect the permanent opening in the centre to occur by the gradual disintegration of the tissue in that region. Transverse sections apparently show that this valve-fold extends dorsad into the heart, cephalad and caudad of the immediate regions of the anterior and posterior ventral orifices.

A still later stage, as shown in the 85 mm. series, demonstrates considerable change in the valves guarding the ventral orifices of the heart both as regards form and general structure. Instead of appearing short and broad in section, as in Fig. 16, they have become long and slender bands of well-differentiated connective tissue, lined with endothelium. As was observed in the 60 mm. series the second or posterior valves are considerably less advanced than the first or anterior pair. In the 85 mm. series the posterior pair are in about the same stage as the anterior pair in the 60 mm. series.

VII. Histogenesis of the Musculi Cordis Caudalis.

In discussing the origin of this muscle it will be necessary to begin with the myotomes, and throughout a comparison of the two is of interest on account of their close relationship both as regards position and innervation.

If a comparison be made of the dorso-ventral extent of the myotomes in the 20 mm. series (Fig. 5, *myo.*) and an 85 mm. stage (Fig. 1, *Myo.*) in which adult conditions are practically reached, it will be seen that the embryonic myotomes in the 20 mm. series must grow considerably, both dorsad and ventrad, before their relative adult dimensions are reached. A higher magnification reveals the central part of the myotome in the 20 mm. series to be much further advanced than is either the dorsal or the ventral end. Here the myoblasts (Fig. 21, *Myo. Myob.*) show the beginnings of

fibrillæ, and the mesenchyme is migrating inward in places to form the internal perimysium (*i. per.*), while both the dorsal and the ventral ends consist only of a mass of undifferentiated myoblasts and mesenchyme, which are continuous with dorso-median and ventro-median masses of greatly thickened mesenchyme. In our study the ventro-median mass of concentrated mesenchyme (Figs. 5 and 19) are of especial interest. It can be traced inward and ventrad to the lateral wall of the caudal heart, and below the heart to be continuous with another conspicuous concentration of mesenchyme that is situated between the two caudal hearts, and is destined to form the membranous stage of the median ventral cartilaginous bar and the median portion of the connective-tissue wall of the caudal heart, while dorsad and ventrad it gradually blends in with the adjacent mesenchyme.

Now the question might be asked, What is the significance of this concentration of mesenchyme, and what will it form? Since cell-division is not particularly active here, it is quite certain that this mesenchyme has collected here, and is migrating inward to form the median ventral cartilaginous bar and the connective-tissue layer of the heart. Moreover, from what we know about the manner of formation of skeletal muscle in general, and the fact that no sharp line of separation can be determined between this mass of mesenchyme and the myotomes, together with the additional fact that the two are innervated by the same nerve, makes it reasonable to suppose that within this mass of mesenchyme there are certain primitive muscle plate cells, that migrated inward along with the mesenchyme, but which, although at present indistinguishable from the mesenchymal cells, have preserved their identity, and will later multiply, arrange themselves outside the heart, and differentiate into m. cordis caudalis myoblasts. A point favouring the presence of early myoblasts in the m. cordis caudalis region is shown by the fact that the third ventral spinal nerve in the heart region, the one which innervates this muscle in the adult, has in the embryo (Fig. 11, *V. Sp. N.*) extended to the

immediate region of this muscle; while the preceding
ventral spinal nerve (Fig. 19, *V. Sp. N.*) has not reached a
level nearly as far ventrad.

In the 27 mm. series (Fig. 20) several important changes have
occurred in the region of the future m. cordis caudalis. The
former reticular appearance of the mesenchyme has to a large
extent disappeared, and in place of it, bunches of embryonic
white fibres have appeared everywhere about and between
the cells; the ventral boundaries of the myotomes (*myo.*)
have descended considerably ventrad, and in place of one
solid mass of concentrated mesenchyme plus a few hypo-
thetical undifferentiated myoblasts, as was described for the
20 mm. series as lying between the myotomes and the caudal
heart, we have in the 27 mm. series (Fig. 20), and more
strongly marked out in sections further cephalad, two quite
distinct areas. First a dense area situated directly laterad of
the caudal heart is composed of a great many round or
slightly spindle-shaped cells separated by a small amount of
embryonic white fibres, and a second thinner area, located
between the first area and the myotomes, consists of but few
cells and more embryonic fibres. The first area will un-
doubtedly form the lateral connective-tissue layer of the
caudal heart and the m. cordis caudalis; although in this
series the myoblasts cannot be detected from the embryonic
connective-tissue cells, still the general shape of the area
itself in transverse section resembles that of the m. cordis
caudalis of a later stage. The second or thinner area repre-
sents nothing more than mesenchyme changing into white
fibrous tissue.

Between the 27 mm. and the 58 or 60 mm. series there is a
considerable gap, resulting naturally in a marked progress in
the histogenesis of the two areas described in the previous
paragraph. The first area has differentiated into the lateral
connective-tissue wall of the caudal heart (Fig. 16, *F. C. T.*)
and into the m. cordis caudalis (Fig. 16, *m. c. c.*); while the
second area is now a mass of embryonic white fibrous tissue,
connecting the m. cordis caudalis with the myotomes. A

closer examination of the m. cordis caudalis will reveal it to be still in a very embryonic condition. Syncytii have formed of the myoblasts, and a few fibrillæ (Fig. 23, *Fib.*) are present; the general state of the muscle is about identical to the myotomes of the 20 mm. series. On the other hand the myotomes of the 60 mm. series have developed well-formed muscle-fibres (Fig. 23, *Myo. F.*).

In the 85 mm. series the first trace of muscle-fibres (Fig. 24, *M.C.F.F.*) have appeared in the m. cordis caudalis. They are still very small, and must increase considerably in diameter before adult conditions are reached. It may be said that in every way the m. cordis caudalis muscle-fibres resemble skeletal muscle rather than cardiac muscle. Turning to the myotomes for comparison we find that the muscle-fibres (Fig. 24, *Myo. F.*) have about reached adult conditions as regards size, but their fibrillæ (*Fib.*) are large and still few in numbers, and must subdivide considerably before an adult state is reached.

The m. cordis caudalis might be said then to have its origin in a very early stage (20 mm.) from myoblasts situated in a condensed mass of mesenchyme that is located between the base of the embryonic myotomes on one side and the embryonic caudal heart and the median ventral cartilaginous bar on the other side. For the most part this mass of cells is migrating mesenchyme that is moving mesad to form the connective-tissue layer for the caudal heart and the median ventral cartilaginous bar, but from our knowledge of the histogenesis of skeletal muscle, which this is, and the fact that it is innervated by the same ventral spinal nerve that innervates the myotomes, and that this nerve reaches the region of the m. cordis caudalis before the myoblasts are differentiated from the adjacent mesenchyme, leads one to believe that certain muscle plate cells must also have migrated inward along with the mesenchyme, and existed for some time in the above-described concentrated mesenchyme, and while they are indistinguishable from the surrounding mesenchymal cells, yet they would have retained

their identity, and eventually will develop into m. cordis myoblasts. The first appearance of the m. cordis caudalis occurs much later than the myotomes, and its fibres never reach anything like the size of the myotome fibres. Figs. 21 to 25 are intended to show the comparative stages of histo-genesis of these two muscles from 20 mm. to 20 cm.

VIII. Summary and General Discussion.

Polistotrema in the adult possesses a distinct system of lymphatics or veno-lymphatics, supplying a region amply furnished with veins. No capillaries were observed connect-ing these vessels with the arteries, but they always contained some red corpuscles, usually great quantities in embryos, and only a few in the adult. In all of the larger and medium-sized embryos the connective tissue outside these vessels was so filled with red corpuscles as to resemble germinative centres, and the vessels themselves appeared to be reservoirs for storing them. On account of the loose state of the endothelium of these vessels it would be an easy matter for an external corpuscle to migrate through the wall, and in this instance it would seem to be a more plausible expla-nation for the appearance of red corpuscles in the veno-lymphatics than to regard them as extravasations from the blood-vessels caused by the rupturing of a delicate wall sepa-rating them. All of the subcutaneous canals are decidedly sinus-like, distinctly recalling the conditions found in the Amphibians. Posteriorly the entire system culminates in two pulsating hearts, which are mere enlargements of the two terminal forks of the caudal vein.

So far as is known, Polistotrema, Myxine, and the eels are the only fish-like vertebrates that possess pulsating caudal hearts. They swim by a snake-like movement, while most fish swim by rapid lateral vibrations of the caudal fin, and would, therefore, not require any specialized pulsating heart, for the motion of the fin against the wall of water would alternately press these caudal sinuses against the

hypural bones and the tail muscles, thus discharging their contents into the caudal veins and functioning as the caudal hearts of Polistotrema.

With the exception of some additions noted in connection with the hæmal lymphatics, the adult veno-lymphatics in the tail region were found to be about the same as Greene and Favaro had described them.

In my youngest stage the arterial system was well formed, while the venous system was in a rather late embryonic state. In the region of the post-gut the caudal vein was in communication with the yolk-sac veins, and near the tip of the notochord a branch on either side anastomosed with a corresponding branch of the caudal artery. Throughout the vein there was everywhere the remains of mesenchymal cavity partitions that had not fully disintegrated at this stage. The caudal vein was described as growing in diameter by the addition to either side of certain isolated mesenchymal spaces, but strange to say, the intersegmental veins were formed as dorsal and ventral sprouts from the lateral surface of the caudal vein.

While the early stages of the formation of the blood-vessels in Polistotrema were not to be had, considerable evidence was gathered in support of a hypothesis that I would like to propose for the origin of the blood-vessels, and which may also hold good for the lymphatics, namely, that the larger longitudinal trunks which follow the main axis of the embryo are developed through the formation and coalescence of certain mesenchymal cavities, while the intersegmental and smaller branches arise as outgrowths from the wall of the main trunks already formed. The process involved in connection with the larger trunks is identical to Lancaster's description of the formation of the blood-vessels in the leach by a vacuolation of the mesenchyme; some of the cells by flattening are going to form the endothelium, and others, by becoming round and increasing in size, are transformed into red corpuscles. In connection with the outgrowths of the intersegmental vessels from the walls of the main trunks it should be noted that all

observations on growing blood-vessels have been confined solely to vessels of this character and not to the longitudinal trunks. The above hypothesis would be somewhat comparable to the development of the nervous system, where you would have the central nervous system being formed from one primary layer of the embryo, and the nerves, which would be analogous to the intersegmental vessels, arising as offshoots from the main stem.

The rate of development of the caudal heart is rather slow : it begins in embryos between 15 and 20 mm. and is taking place in embryos of 85 mm. This construction stage doubtless occupies a period of several months.

The caudal heart of Polistotrema differs from the lymph-sacs of birds and mammals in that it never loses its primary connection with the vein and acquires a second communication. In the adult it is simply an expansion of the vein.

Concerning the construction of the caudal hearts of Polistotrema, they have been described as having been excavated out of the mesenchyme dorsad and ventrad of the anterior ends of the two forks of the caudal vein, through the disintegration of the walls of large mesenchymal cavities, which for the most part are connected with each other and the vein, and which were originally formed from the isolated mesenchymal spaces after the manner set forth on p. 338. The process involved in the formation of these isolated mesenchymal spaces is identical to what Lancaster found in the leach, namely, a vacuolation of the mesenchyme by the disintegration of certain of the mesenchymal cell processes, and the flattening of some mesenchymal cells to become endothelium and a rounding of others to become red corpuscles.

In connection with the 20 mm. series A, the possibility was noted of the proximal ends of three so-called dorsal embryonic intersegmental veins or capillaries contributing in a very limited extent to the formation of the caudal hearts.

It will be seen, then, that the development of the caudal hearts in Polistotrema as I have interpreted them is in harmony with what Stromsten found in the turtle, but at

variance with a considerable recent work on the development of the lymph-sacs in the higher vertebrata.

Closely associated with the early construction of the caudal hearts occurs the formation of the ventral trunk, at first paired, and each consisting of a longitudinal chain of isolated mesenchymal cavities, which later become connected with each other and the caudal heart.

The paired valves guarding the orifices of the anterior and posterior communications between the ventral veno-lymphatic trunk and the caudal heart take their origin from a migration inward and dorsad of the mesenchymal wall on either side of the orifice.

The endothelium has already been described as coming from the flattening of some of the border-cells of the larger mesenchymal cavities, and in like manner the connective-tissue endocardium comes from a concentration and differentiation of the mesenchyme about the endothelial lining.

There is no true myocardium in the caudal heart of Polistotrema, but the functional myocardium, the M. cordis caudalis, arises from myoblasts in the centre of a mass of concentrated mesenchyme situated between the base of the myotomes and the caudal heart that is migrating inward to form the median ventral cartilaginous bar and the connective-tissue layer of the caudal heart. Since the muscle-fibres of the M. cordis caudalis are true skeletal muscle, their myoblasts are supposed to have originated from primitive muscle plate cells that have migrated inward with the surrounding mesenchyme. It was demonstrated that the ventral spinal nerve which innervated this muscle also supplied the myotomes, and in the early embryos reached the area of the future M. cordis caudalis long before its myoblasts were distinguishable from the adjacent mesenchyme.

In conclusion it might be said that my studies thus far indicate that the most primitive form of a lymphatic system are veins that function for both lymphatics and veins. Hence it would be expected that ontogeny would repeat the phylogeny of the lymphatics, and instead of having their

origin directly from veins, that they would begin exactly as the
veins did, namely, by the vacuolation of the original mesen-
chyme.   In one of the primitive vertebrates, Polistotrema,
I have described these vessels as veno-lymphatics, by which
is meant a system of vessels that is more closely related to
the veins than the lymphatics of the higher vertebrata are.   In
a later communication I expect to show that the main factor
in the construction of the veno-lymphatic system is the same
as was described for the caudal hearts, namely, the formation
and union of certain mesenchymal spaces.

The following paper was completed August 28th, 1912, and a
considerable of the later work on it was done at the Institute
of Anatomy of the University of Minnesota.

IX. EXPLANATION OF PLATES 19–21,

Illustrating Mr. William F. Allen's paper on " Studies on the
Development of the Veno-Lymphatics in the Tail Region
of Polistotrema (Bdellostoma) Stouti.   First
Communication : Formation of the Caudal Hearts."

[With the exception of Figs. 4 and 4 A, which are from dissections of
an adult and a diagrammatic reconstruction, all figures are from trans-
verse sections or from accurate graphic reconstructions.

In the transverse sections all vessels were drawn in outline as tubes,
primary mesenchyme as cells with processes or as simple dots in the
low power drawings. A concentration or centralisation of mesenchyme
to form an organ is set forth by an increased number of these cells or
dots. A differentiation of mesenchyme into connective tissue is shown
by short irregular lines running in all directions. Muscle, when seen
by low power, is indicated by fine dots, and cartilage by much coarser
dots. All of the outlines, including the cells and their protoplasmic
processes, were made with the aid of a camera lucida.

Concerning the graphic reconstructions, most accurate drawings
were made of each, or part of each section, with a magnification of
100, 200, or 400 diameters. In case of the low-power reconstruction
drawings, either additional high-power drawings were made, or careful
comparisons were made with the high power, of each structure drawn.
At the outset, a base line from which all measurements were taken
was established by drawing a line at the level of the ventral surface

of the notochord at right angles to a median sagittal plane, and this base-line was added to each successive drawing by accurately placing the second or following drawing over the first above a plate of glass illuminated from below. In every reconstruction this base line is indicated by the dotted line (*B. L.*), and from it all the vessels and organs were measured off with dividers and plotted on ruled mm. paper.

The caudal artery in all the reconstructions is represented by circular cross-lines, the spinal cord by oblique cross-lines, the caudal vein and caudal heart by outlines only; the notochord, when completely reconstructed, by coarse dots, otherwise simply as a line to indicate its lower border. Certain mesenchymal spaces that are taking part in the formation of the caudal heart are indicated in outline. Of these there are two kinds: those indicated by small (*c.*) have not at this stage attained connection with the caudal vein; and another class, indicated by capital (*C.*), that have gained one or more connections with the caudal vein. When these cavities are located above or below or in front (laterad) of the caudal vein or other organ they are indicated in outline, but when they lie median to them they are indicated in dotted outline. Also any vessel lying median to an organ or other vessel is shown in dotted lines. A layer of mesenchyme separating the caudal vein from a mesenchymal space or two caudal heart mesenchymal spaces, or the caudal heart from the ventral veno-lymphatic trunk, is represented by a solid black space.]

LIST OF ABBREVIATIONS USED IN THE FIGURES.

A. or *P.* prefixed to an abbreviation signifies anterior or posterior, and *R.* or *L.* right or left. Embryonic structures are indicated by small letters, and adult or nearly adult by capital letters.

a. In reconstructions, marks the place of bifurcation of the caudal artery. *A. L. Hæ. T.* (1). Anterior fork of the posterior longitudinal hæmal lymphatic trunk. *b.* In reconstructions, marks the place of union of the two caudal veins. *B. L.* In reconstructions, base line from which all measurements were made. *B. V.* Blood-vessel. *C.* Caudal heart mesenchymal space that had assumed connection with the caudal vein or with other caudal heart mesenchymal spaces. *c.* Primitive caudal heart mesenchymal space that had not at this stage assumed connection with the caudal vein or with other caudal heart mesenchymal spaces. *Cau. A.* Caudal artery. *Cau. V.* Caudal vein. *C. T.* Connective tissue. *c. t.* Embryonic connective tissue. *d.* In Fig. 4 A, large veno-lymphatic sinus lying on the lateral surface of the last two mucous sacs. *D. A.* Dorsal fin-ray artery. *D. Bar.* Median dorsal

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cartilaginous bar or basal fusion of the dorsal fin-rays or radials. *d. bar.* Massing of the mesenchyme to form the median dorsal bar. *Dep.* In Fig. 7 certain depressions in the lateral wall of the caudal vein, which doubtless represents the remains of an earlier embryonic condition of the vein. *D. R.* Dorsal fin-radials or rays. *D. S.* Dorsal fin-ray veno-lymphatic canals or sinuses. *D. V.* Dorsal veno-lymphatic trunk. *d. v.* Above in embryonic condition. *Ep.* Epidermis. *e. per.* Embryonic external perimysium. *F. C. T.* Fibrous connective tissue. *Fib.* Muscle-fibrillæ. *Hæ. T.* Intersegmental or hæmal lymphatic canal. *Ints. A.* Intersegmental artery. *Ints. V.* Intersegmental vein. *i. per.* Embryonic internal perimysium. *L. Cau. A.* Left caudal artery. *L. Cau. H.* Left caudal heart. *l. cau. h.* Above in embryonic condition. *L. Cau. H.* (1). Anterior portion of the left caudal heart. *L. Cau. V.* Left caudal vein. *L. Hæ. T.* Longitudinal hæmal lymphatic trunk or trunks. *L. Hæ. T.* (1). Posterior portion of the longitudinal hæmal lymphatic trunk. *L. L. Hæ. T.* Left longitudinal hæmal lymphatic trunk. *L. L. Hæ. T.* (1). Left branch of the posterior portion of the longitudinal hæmal lymphatic trunk. *L. L. S.* Left lateral veno-lymphatic sinus or trunk. *l. l. s.* Above in embryonic condition. *L. L. S. C.* Left lateral sinus mesenchymal cavity. *L. L. S. Con.* Left lateral sinus connection with the ventral trunk. *L. Proc.* Lateral process of the median ventral cartilaginous bar. *l. proc.* Above in embryonic condition. *L. V. V.* Left ventral veno-lymphatic trunk. Posterior portion homologous to caudal trunk of the higher fishes. *l. v. v.* Above in embryonic condition. *M. C. C.* Musculi cordis caudalis or myocardium of Favaro. *m. c. c.* Above in embryonic condition. *M. C. C. F.* Musculis cordis caudalis fibre. *M. C. C. Myob.* Musculi cordis caudalis myoblast. *Mes.* Mesenchyme. *M. Sac.* Mucous sac. *My.* Myelon or spinal cord. *Myo.* Myotomes. *myo.* Above in the process of formation. *Myob.* Myoblasts. *Myo. F.* Myotome muscle fibre. *Myo. Myob.* Myotome myoblast. *Nc.* Notochord. *O.* (1). First or anterior orifice between the ventral veno-lymphatic trunk and the caudal heart. *o.* (1). Probable point where the anterior orifice between the ventral trunk and the caudal heart will later occur. *O.* (2). Second or posterior communication between the ventral veno-lymphatic trunk and the caudal heart. *o.* (2). Probable place of the posterior communication between the ventral trunk and the caudal heart. *P.* In reconstructions, partition of mesenchyme separating two parts of the caudal vein or heart. Possibly the remains of a wall separating two cavities. *R. C.* Red corpuscle. *R. Cau. A.* Right caudal artery. *R. Cau. H.* Right caudal heart. *r. cau. h.* Above in embryonic condition. *R. Cau. V.* Right caudal vein. *R. L. Hæ. T.* Right longitudinal hæmal lymphatic trunk. *R. L. Hæ. T.* (2). Right branch of the posterior portion of the longitudinal hæmal lymphatic trunk. *R. L. S.* Right

lateral veno-lymphatic sinus or trunk. *r. l. s.* Above in embryonic condition. *R. V. V.* Right ventral veno-lymphatic trunk. Posterior portion homologous to the caudal trunk of the higher fishes. *r. v. v.* Above in embryonic condition. *S. Gan.* Spinal ganglion. *V. A.* Ventral or anal fin-ray artery. *Val.* (1). Valves guarding the anterior orifice between the ventral veno-lymphatic trunk and the caudal heart. *val.* (1). Above in the process of formation. *V. Bar.* Median ventral cartilaginous bar or basal fusion of the ventral or anal fin-radials. *v. bar.* Above in the process of formation. *Val. F.* Valve fold ·extending up into the caudal heart from the sides of the orifice between the heart and the ventral trunk. *V. L. Hæ. T.* Ventral or inferior longitudinal hæmal lymphatic trunk. *V. R.* Ventral or anal fin-radials. *v. r.* Above in the process of formation. *V. S.* Ventral or anal fin veno-lymphatic canals or sinuses. *v. s.* Above in embryonic condition. *V. Sp. N.* Ventral spinal nerve ramus. *V. V.* Ventral veno-lymphatic trunk. Posterior portion homologous to the caudal trunk of the higher fishes. *v. v.* Above in the process of formation. *Z.* Indicates posterior ending of the embryonic caudal heart in reconstructions as located by the crossing of the third spinal nerve in the heart region.

Fig. 1.—Represents a transverse section through the tail region of an 85 mm. Polistotrema embryo; cut passing through the anterior region of the caudal hearts and viewed from the rear. This section shows the enormous lateral veno-lymphatic sinuses or trunks together with their dorsal and anal fin branches, the longitudinal dorsal and ventral veno-lymphatic trunks, and the caudal hearts in transverse section. Observe the left lateral sinus emptying into the left ventral trunk, and the right ventral trunk communicating with the right caudal heart; its orifice being guarded by two valves opening into the heart. It should be mentioned that the original section showed the great lateral veno-lymphatic sinus to be completely distended by blood-corpuscles. × 25.

Fig. 2.—Is from the same series as Fig. 1, but 390 microns cephalad. Less than one half of the section is figured. This section passes through the caudal hearts as they are about to terminate in the caudal vein. Note especially that the longitudinal hæmal lymphatic trunks have united to form a single posterior transverse stem, which passes ventrad and divides into anterior and right and left branches that empty separately into the ventral veno-lymphatic trunk after encircling the median ventral bar. × 25.

Fig. 3.—From same series as above. Section is taken several slides cephalad of Fig. 2. and only the main longitudinal hæmal vessels are shown in relation to each other and to the notochord. Observe the tendency for the longitudinal hæmal lymphatic trunks to divide into two

portions and for the ventral portion to receive an intersegmental branch. × 25.

Fig. 4.—Is from a dissection of the left caudal heart and the adjacent lymphatic and blood-vessels, excepting the lateral veno-lymphatic sinus and its dorsal and ventral or anal fin branches of an adult Polistotrema are omitted. Any attempt to represent the enormous lateral sinus in this drawing would necessarily obliterate everything that was more deep-seated. It should, however, be stated that both the dorsal and ventral canals had frequent communications with the lateral sinuses. × 2.

Fig. 4 A.—Diagrammatic reconstruction of the hæmal lymphatic system of a 20 cm. Polistotrema in the region directly cephalad of the caudal hearts as seen from the left side.

Fig. 5.—Transverse section through the tail of a 20 mm. Polisto-trema embryo, series B. Section passes through the anterior end of the caudal hearts, which are in the process of formation. Primarily it is intended to show the position of the embryonic caudal hearts and their relation to other developing structures, especially to the embryonic lateral sinus, which at this stage consists of several mesenchymal spaces uniting in front and behind, but which in a later stage will be one con-tinuous and much larger cavity. At this stage none of the veno-lymphatic trunks have gained connection with each other or with the caudal heart. Note the large mesenchymal cavity (C.), which in the next section caudad (Fig. 13) connects with the caudal vein. Also a more ventral cavity ($l.v.v.$) is shown that has at this stage no connection with the caudal vein, and which for reasons stated in the text I take to be the beginning of the posterior portion of the ventral veno-lymphatic trunk. × 50.

Fig. 6.—Graphic reconstruction of the left caudal artery, left caudal vein, left embryonic caudal heart, and the left embryonic ventral veno-lymphatic trunk of a 20 mm. Polistotrema embryo, series B, as seen from the left side. Near the tip of the spinal cord a space of 640 microns has been left out of the reconstruction. Behind this the posterior ending of the notochord, spinal cord, and the union of the left caudal vein in the left caudal artery is clearly shown. Of especial sig-nificance are the mesenchymal spaces, (c.) and (C.), which are undoubtedly the main factors in the formation of the caudal hearts. × 200.

Fig. 7.—Identical reconstruction of the same structures of another 20 mm. Polistotrema embryo, series A, as Fig. 6, except that this re-construction is not carried as far caudad. It should be noted in the caudal heart region that there is a direct anastomosis of an interseg-mental vein with an intersegmental artery. If the ventral veno-lymphatic trunk is present it is represented by a small mesenchymal

space (*l. v. v.*). In some respects series A is more embryonic than series B. × 200.

Fig. 8.—Graphic reconstruction of the left caudal artery, vein, heart, and the ventral and hæmal veno-lymphatic systems of a 60 mm. Polistotrema. Posteriorly the caudal heart is shown to be still in the process of formation, but in the main it has assumed adult conditions. Likewise the ventral veno-lymphatic trunk has attained practically adult conditions. Two connections are established with the left caudal heart, each of which is guarded by a pair of lateral valves, and since this is a median reconstruction, it passes between them, so that they are not shown in this figure. In the region immediately in front of the caudal hearts the longitudinal hæmal lymphatic trunks proper are practically the same as in the adult, but no trace of the ventral longitudinal hæmal lymphatic trunk has apppeared in this stage. × 50.

Fig. 9.—Graphic reconstruction of a portion of the notochord, the caudal artery and the left caudal artery, the caudal vein and the left caudal vein, the embryonic caudal heart and the embryonic ventral veno-lymphatic trunk of a 25 mm. Polistotrema. It should be noted that the caudal heart is fairly well formed anteriorly, but posteriorly it is in an early stage of formation, showing very conclusively that it is a gradual process, taking place from cephalad to caudad. This process is discussed at length in the text. Considerable progress is also shown in connection with the development of the ventral veno-lymphatic trunk over the 20 mm. reconstructions. An identical mesenchymal cavity (*l. v. v.* (1)) is shown, but it has increased in size and obtained connection with the embryonic caudal heart. Also additional mesenchymal spaces have put in their appearance in a direct line posterior to this, and in one place (*o.* (2)) there is a collection of small cavities, indicating possibly the place where the second or posterior connection with the caudal heart will occur. × 100.

Fig. 10.—Identical reconstruction of the same structures of a 27 mm. Polistotrema as the previous reconstruction of the 25 mm. Polistotrema. It, however, shows considerable advancement, although from its centre to its posterior border it is in more or less embryonic condition. Two large mesenchymal cavities (*C.*) that have gained connection with the caudal heart and vein are very conspicuous. From a lateral view the posterior might readily be taken for an intersegmental vein, or a degenerate intersegmental vein that was taking part in the formation of the caudal heart, but, as was shown in the text, its extreme median position is against this hypothesis. Note the marked and regular progress in the formation of the ventral veno-lymphatic trunk. Cavities (*l. v. v.* (1), (2)) have coalesced in one long cavity and cavities (*l. v. v.* (3), (4)) have also united, and both of these large cavities have

attained connection with the caudal heart. In two places they have
gained connection with a mesenchymal space belonging to the embryonic
lateral sinus, and in one place a direct connection has been established
with the left lateral sinus.   × 100.

Fig. 11.—Transverse section through the extreme posterior portion
of the left embryonic caudal heart of a 20 mm. Polistotrema, series
B. Its exact position is shown by the line 11 in Fig. 6. Note that the
endothelium of the caudal vein is but little differentiated, and the cells
and their processes cannot be told from the mesenchymal cells imme-
diately outside that will be concerned in the formation of connective
tissue and the median cartilaginous bar. Of especial interest is the
primitive mesenchymal space (c.), which has not at this stage gained
connection with the caudal vein. but which in a later stage would
doubtless take part in the formation of the caudal heart.   × 225.

Fig. 12.—Transverse section through the median portion of the
embryonic caudal hearts of the same 20 mm. embryo as Fig. 11, but taken
200 microns cephalad; the exact position of the section is shown by the
line 12 in Fig. 6. In the left heart note the large ventral and the small
dorsal mesenchymal spaces, which have attained connection with the
left caudal vein. Also the posterior tip of another cavity is seen laterad
of the dorsal cavity, which expands further caudad and terminates in
the left caudal vein. The embryonic right caudal heart consists of
several ventral cavities (C.), which unite further caudad and cephalad in
larger cavities that terminate in the vein. Also where the red corpuscle
(R. C.) is suspended in the mesenchyme there is evidence of the begin-
ning of a primitive mesenchymal space. In short, this section shows
most all of the various stages of the mesenchymal spaces that go to
make up the caudal heart. At every point the endothelium gives
evidence of being differentiated mesenchyme, and the termination of
the mesenchymal spaces in the caudal vein have the appearance of
acquiring secondary connections with it rather than out-budding from it.
× 225.

Fig. 13.—Transverse section through the extreme anterior end of the
left embryonic caudal heart of the same embryo as Figs. 11 and 12, being
taken 120 microns cephalad of Fig. 12. and its exact position in Fig. 6 is
shown by the line 13. Observe that the orifice of cavity (C.) has the
appearance of having acquired connection with the vein by the breaking
down of the wall separating them.   Also what I take to be the first
mesenchymal space of the ventral veno-lymphatic trunk (l. v. v. (1)) is
shown in cross-section. It is full of red corpuscles, and the loose mesen-
chyme above is possibly indicative of the place where the first or anterior
communication with the caudal heart will occur. A comparison of the
endothelium of the various regions with mesenchyme is of interest.
× 225.

Fig. 14.—Transverse section through the centre of the left embryonic
caudal heart of a 20 mm. Polistotrema, series A. Its exact position
is shown by the line 14 in Fig. 7. Observe the direct anastomosis of a
so-called intersegmental vein with an intersegmental artery and the
termination of a mesenchymal space (C.) in the vein. This shows that
an intersegmental vein may take some part in the formation of the
caudal heart. A comparison of the adjacent structures is also of
interest. × 130.

[Figs. 15 to 18 are introduced to show the nature of the orifice between
the ventral veno-lymphatic trunk and the caudal heart, the mode of
development of the valves guarding this orifice, and something of the
structure of these vessels in the various stages.]

Fig. 15.—Transverse section through the anterior orifice between the
embryonic ventral veno-lymphatic trunk and the left embryonic caudal
heart of a 27 mm. Polistotrema. Note that the endothelium is
barely differentiated from the mesesenchyme and that no valves have
appeared, but that there is a proliferation of cells [*val.* (1)], which may
be the forerunner of the valves. × 225.

Fig. 16.—Transverse section through the two valves guarding the
first or anterior orifice between the ventral veno-lymphatic trunk and
the left caudal heart of a 60 mm. Polistotrema. At a glance it will
be seen that the valves have almost reached adult conditions, that they
are formed by a proliferation and massing inward and dorsad of the
mesenchyme between these two structures. At this stage the mesen-
chyme of the valves has not become differentiated into fibrous con-
nective tissue lined with endothelium. These valves have made fully
twice as much advance as the valves guarding the second or posterior
orifice. It will be seen that the caudal heart is lined with endothelium,
outside of which the mesenchyme has differentiated into a sort of
fibrous connective tissue. The musculi cordis caudalis is well defined
and fibrillæ are making their first appearance in its myoblasts. × 225.

Figs. 17 and 18 are transverse sections through the valves guard-
ing the orifice of the ventral veno-lymphatic trunk with the right
caudal heart of the same embryo as Fig. 16, and the two figures are
from adjoining sections. Both show that the valves guarding this
orifice are decidedly more embryonic than is the case with the opposite
or left caudal heart-valves. The valves are here represented as two
masses of mesenchyme that have grown inward and upward from the
lateral sides of the orifice, having fused in some places and in others an
open communication remains. In Fig. 17 the orifice is on the left side,
in the next section cephalad (Fig. 18) there is no orifice, and in the next
section cephalad of Fig. 18 the orifice is found to be on the right side.
× 225.

[Figs. 19–25 have been introduced to show the comparative rate of growth and histogenesis of the myotomes and the musculi cordis caudalis.]

Fig. 19.—Transverse section through the lower portion of the embryonic myotomes and the embryonic musculi cordis caudalis of a 20 mm. Polistotrema, series A. In this stage the myoblasts in the ventral end of the myotomes are not as far advanced as they are further dorsad, having no sign of fibrillæ, and no sign of perimysium has thus far appeared. It is continuous, however, with a greatly thickened mass of mesenchyme, which has probably migrated inward to form the connective tissue for the caudal heart and other structures, and in this greatly thickened mass of mesenchyme there may be musculi cordis caudalis myoblasts (?) indistinguishable from the adjacent mesenchyme, and which may have migrated in from the mesoblastic somites with the mesenchyme.   × 130.

Fig. 20.—From an identical transection to Fig. 19, but from a 27 mm. embryo, showing the same structures slightly advanced. Note the formation of the external perimysium and the separation of the myotomes from the thickened mass of mesenchyme described above, and that the protoplasmic processes of this mesenchyme has undergone a change possibly preparatory to the forming of fibrous connective-tissue fibres, while the m. cordis caudalis boundaries are outlined.   × 130.

Fig. 21.—(1) On the left, a transection of a small portion of the embryonic myotomes taken from about the centre. (2) On the right, similar portion of the musculi cordis caudalis in transection. Note that the fibrillæ and internal perimysium have made their appearance in the myotomes, but not in the m. cordis caudalis.   × 225.

Fig. 22.—Identical to Fig. 21, but from a 27 mm. series. External perimysium has appeared in the myotomes and a slight change has occurred in the intercellular spaces of the embryonic m. cordis caudalis.   × 225.

Fig. 23.—Identical to Fig. 21, but from a 60 mm. embryo. The muscle-fibres of the myotomes are fairly well developed, and fibrillæ are making their first appearance in the myoblasts of the m. caudis caudalis.   × 225.

Fig. 24.—Identical to Fig. 21, but from an 85 mm. embryo. The muscle-fibres of both the myotomes and the m. cordis caudalis are well-formed. Note the presence of Cohnheim's areas in the former and the much larger size of the fibres in the myotomes.   × 225.

Fig. 25.—Identical to fig. 21, but from a small adult 10 cm. long. Both fibres have attained adult conditions. Note how much smaller the m. cordis caudalis fibre is than the myotome fibre. The m. cordis caudalis is also extremely rich in blood-vessels.   × 225.